NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

TETRAHYDROFURAN

(CAS NO. 109-99-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

June 1998

NTP TR 475

NIH Publication No. 98-3965

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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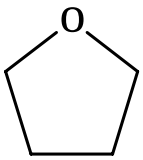
ABSTRACT



TETRAHYDROFURAN

CAS No. 109-99-9

Chemical Formula: C_4H_8O Molecular Weight: 72.10

Synonyms: Butylene oxide; cyclotetramethylene oxide; diethylene oxide; 1,4-epoxybutane; furanidine; hydrofuran; oxacyclopentane; oxolane; tetramethylene oxide

Tetrahydrofuran is used as a reaction medium for Grignard and metal hydride reactions; in the synthesis of butyrolactone, succinic acid, and 1,4-butanediol diacetate; in the fabrication of articles for packaging, transporting, and storing of foods; as a solvent for dyes and lacquers; and as a chemical intermediate in polymerization solvent for fat oils, unvulcanized rubber, resins, and plastics. Tetrahydrofuran is also an indirect food additive when it is in the contact surface of articles intended for use in food processing. Tetrahydrofuran was nominated for study because of the potential for occupational exposure in humans. Male and female F344/N rats and B6C3F$_1$ mice were exposed to tetrahydrofuran (approximately 99% pure) by inhalation for 14 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, mouse bone marrow cells, and mouse peripheral blood erythrocytes.

14-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were exposed to 0 (chamber control), 66, 200, 600, 1,800, or 5,000 ppm tetrahydrofuran by inhalation, 6 hours per day, 5 days per week, for 14 weeks. All rats survived until the end of the study. Final mean body weights and body weight gains of exposed groups of male and female rats were similar to those of the chamber controls. Immediately after exposure, male and female rats in the 5,000 ppm groups exhibited ataxia.

Hematologic and serum chemistry changes were minimal, with most values falling within physiologic ranges. Absolute and relative thymus and spleen weights of male and female rats exposed to 5,000 ppm were significantly less than those of the chamber controls. Absolute and relative liver weights of female rats exposed to 5,000 ppm were significantly greater than those of the chamber controls. Increased incidences of minimal to mild hyperplasia of the forestomach were observed in male and female rats exposed to 5,000 ppm. Minimal suppurative inflammation was associated with forestomach hyperplasia in two male and four female rats exposed to 5,000 ppm.

14-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to 0, 66, 200, 600, 1,800, or 5,000 ppm

tetrahydrofuran by inhalation, 6 hours per day, 5 days per week, for 14 weeks. Two male mice exposed to 5,000 ppm died during weeks 2 and 8 of the study; one male mouse from the 5,000 ppm group was killed in a moribund state during week 4. All female mice survived until the end of the study. The final mean body weights and body weight gains of all exposed groups of male mice were similar to those of the chamber controls. The final mean body weight and body weight gain of the 5,000 ppm female mice were significantly greater than those of the chamber controls. Male and female mice exposed to 1,800 or 5,000 ppm were observed to be in a state of narcosis (described by stupor) during exposure periods. Mice exposed to 1,800 ppm were fully awake and alert immediately after exposure; however, mice exposed to 5,000 ppm required up to 2 hours for recovery.

Absolute and relative liver weights of male mice exposed to 600 ppm or greater and of female mice exposed to 1,800 or 5,000 ppm were significantly greater than those of the chamber controls. Absolute and relative thymus weights of male mice exposed to 600, 1,800, or 5,000 ppm were significantly less than those of the chamber controls. The incidences of minimal to mild centrilobular cytomegaly of the liver in male and female mice exposed to 5,000 ppm were significantly greater than those in the chamber controls. The adrenal glands of all female mice exposed to 5,000 ppm had mild degeneration of the X-zone of the innermost cortex. Uterine atrophy was observed in all female mice exposed to 5,000 ppm.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were exposed to 0, 200, 600, or 1,800 ppm tetrahydrofuran by inhalation, 6 hours per day, 5 days per week, for 105 weeks.

Survival and Body Weights

Survival rates and mean body weights of male and female rats exposed to tetrahydrofuran were similar to those of the chamber controls.

Pathology Findings

The incidences of renal tubule epithelial adenoma or carcinoma (combined) in exposed males occurred with a positive trend, and the incidences in 600 and 1,800 ppm males exceeded the historical range for chamber controls in 2-year NTP inhalation studies.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were exposed to 0, 200, 600, or 1,800 ppm tetrahydrofuran by inhalation, 6 hours per day, 5 days per week, for 105 weeks.

Survival, Body Weights, and Clinical Findings

After week 36, the survival of male mice exposed to 1,800 ppm was significantly less than that of the chamber controls. Mean body weights of male and female mice exposed to tetrahydrofuran were similar to those of the chamber controls throughout the study. Male mice exposed to 1,800 ppm were observed to be in a state of narcosis during and up to 1 hour after the exposure periods.

Pathology Findings

The incidences and multiplicity of hepatocellular neoplasms were significantly greater in female mice exposed to 1,800 ppm than in the chamber controls. The incidence of nephropathy in 200 ppm male mice was significantly greater than that in the chamber control group. Male mice exposed to 1,800 ppm had significantly greater incidences of nonneoplastic lesions of the urogenital tract than did the chamber controls. The incidences of inflammation of the penis and urethra and necrosis of the urethra in 1,800 ppm males were slightly greater than those in the chamber controls; these may have been secondary effects of ascending urinary tract infection.

GENETIC TOXICOLOGY

Tetrahydrofuran showed little evidence of mutagenic activity in a variety of *in vitro* and *in vivo* assays. It was not mutagenic in *S. typhimurium*, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. These *in vitro* tests were conducted with and without exogenous metabolic activation from induced liver S9 enzymes. No increase in sex-linked recessive lethal mutations was detected in germ cells of male

D. melanogaster exposed to tetrahydrofuran by feeding or injection. Results of *in vivo* assays for induction of chromosomal aberrations and sister chromatid exchanges in mouse bone marrow cells were negative. A micronucleus test in male and female mice exposed to tetrahydrofuran for 14 weeks showed no significant increases in the frequency of micronucleated erythrocytes in peripheral blood of female mice, but in male mice, analysis of micronucleated normochromatic erythrocyte levels revealed a small increase above baseline that was concluded to be equivocal.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of tetrahydrofuran in male F344/N rats based on increased incidences of renal tubule adenoma or carcinoma (combined). There was *no evidence of carcinogenic activity* of tetrahydrofuran in female F344/N rats exposed to 200, 600, or 1,800 ppm or male $B6C3F_1$ mice exposed to 200, 600, or 1,800 ppm. There was *clear evidence of carcinogenic activity* of tetrahydrofuran in female $B6C3F_1$ mice based on increased incidences of hepatocellular neoplasms.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tetrahydrofuran

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations	Chamber control, 200, 600, or 1,800 ppm	Chamber control, 200, 600, or 1,800 ppm	Chamber control, 200, 600, or 1,800 ppm	Chamber control, 200, 600, or 1,800 ppm
Body weights	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group
2-Year survival rates	12/50, 6/50, 5/50, 6/50	25/50, 25/50, 26/50, 26/50	32/50, 31/50, 28/50, 12/50	29/50, 33/50, 26/50, 32/50
Nonneoplastic effects	None	None	None	None
Neoplastic effects	Kidney: renal tubule adenoma or carcinoma (1/50, 1/50, 4/50, 5/50)	None	None	Liver: hepatocellular adenoma (12/50, 17/50, 18/50, 31/48); hepatocellular carcinoma (6/50, 10/50, 10/50, 16/48); hepatocellular adenoma or carcinoma (17/50, 24/50, 26/50, 41/48)
Level of evidence of carcinogenic activity	Some evidence	No evidence	No evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537			
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Mouse bone marrow *in vivo*:	Negative			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Mouse bone marrow *in vivo*:	Negative			
Sex-linked recessive lethal mutations				
Drosophila melanogaster:	Negative			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:	Equivocal in male mice; negative in female mice			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (I) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on tetrahydrofuran on 11 December 1996 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 11 December 1996, the draft Technical Report on the toxicology and carcinogenesis studies of tetrahydrofuran received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.S. Chhabra, NIEHS, introduced the toxicity and carcinogenesis studies of tetrahydrofuran by discussing uses of the chemical and rational for study, describing the experimental design, reporting on survival and body weight effects, and commenting on chemical-related neoplastic lesions in male rats and female mice. The proposed conclusions were *some evidence of carcinogenic activity* of tetrahydrofuran in male F344/N rats, *no evidence of carcinogenic activity* in female F344/N rats and male B6C3F$_1$ mice, and *clear evidence of carcinogenic activity* in female B6C3F$_1$ mice.

Dr. Brown, a principal reviewer, agreed with the proposed conclusions. In view of the central nervous system symptoms present in both species, he suggested that a comment be added to the discussion section regarding histologic studies of the central nervous system in both rats and mice in the 2-year studies. Dr. Chhabra agreed, noting that as with many solvents these are nonspecific types of effects. He said that the United States Environmental Protection Agency has asked industry to submit data on acute neurobehavioral toxicity studies.

Dr. Ryan, the second principal reviewer, agreed with the proposed conclusions. She had initial reservations about the strength of renal neoplasm data in male rats but after reviewing historical control incidences of these neoplasms in inhalation studies she was persuaded that the level of evidence was appropriate.

Dr. LeBoeuf, the third principal reviewer, did not agree with the proposed conclusions in male rats and female mice. He stated that there was a marginal treatment-related effect in male rats and, further, a treatment-related effect on renal tubule hyperplasia was not observed, which he considered *equivocal evidence of carcinogenic activity*. He said that a detailed step-sectioning of the kidneys would be appropriate. Dr. Chhabra said that step sections were not called for as the staff was confident of the proposed conclusion of *some evidence of carcinogenic activity* based on the number of neoplasms in exposed animals contrasted with the historical rate. Dr. J.R. Hailey, NIEHS, noted that in almost all previous studies in which step sections were performed, ethylbenzene being the exception, if the level of evidence was *some evidence*, the additional sectioning did not support a change to *clear evidence*. Dr. LeBoeuf said that he would defer further comments on the conclusions in female mice until clarification of possible confounding effects of *Helicobacter hepaticus* present in the livers of mice.

Dr. LeBoeuf asked for further discussion around the relevance or interpretation of neoplasm induction when survival is so poor, and perhaps not attributable to neoplasm induction. Dr. J.K. Haseman, NIEHS, pointed out that in this study, male rat survival was low in all groups, exposed and controls alike. In response to a comment from Dr. W.T. Allaben, NCTR, Dr. Haseman agreed that high body weight could be a factor contributing to the overall poor survival in male rats. Dr. L.G. Hart, NIEHS, read comments into the record from Dr. F. Mirer, NTP Board member. Dr. Mirer said that a structural analogy of tetrahydrofuran to furan is misplaced; rather it should be diethyl ether. He commented that, because there were no differences between exposed and chamber control animals in weight gains or mortality, a higher exposure might have been tolerated increasing the sensitivity for detecting carcinogenic effects. Dr. Chhabra said that narcosis was induced at 1,800 ppm, precluding giving higher exposure concentrations.

Dr. Brown moved that the Technical Report on tetrahydrofuran be accepted with revisions discussed and the conclusions as written for male rats, *some evidence of carcinogenic activity*, for female rats and male mice, *no evidence of carcinogenic activity*, and for female mice, *clear evidence of carcinogenic activity*. Dr. Ryan seconded the motion, which was accepted unanimously with seven votes.